A NEWSLETTER OF THE NEWBORN SCREENING PROGRAM AND THE NEWBORN SCREENING LABORATORY
UTAH DEPARTMENT OF HEALTH

GALACTOSEMIA

SUMMER 2004

MAKING THE MOST OF THIS NEWSLETTER

This issue of the newborn screening newsletter is devoted entirely to galactosemia, one of the four conditions screened on the Utah newborn screening panel.

Classical galactosemia is a rare condition, occurring once in every 60,000 births. Besides detecting infants with classical galactosemia, infants with other treatable galactosemia conditions and infants who are carriers of galactosemia or a galactosemia variant are identified by newborn screening. This increases the chance that you could care for a patient with a positive galactosemia screen.

After reviewing this newsletter you should have a better understanding of what galactosemia is, why it is included on the newborn screening panel, and what a positive result will mean for the families you care for. Taking time to familiarize yourself with this condition will prepare you for the time when galactosemia shows up in your practice.

Thank you to the galactosemia experts in Utah who contributed articles for the galactosemia newsletter! We thank you for lending your expertise and time. And a special thank you to the hospitals, midwives, and medical homes of Utah whose diligence in collecting newborn screens saves the lives of our infants.

Stay tuned for upcoming newsletters on other conditions screened for on the Utah newborn screening panel!

INSIDE THIS ISSUE:

COLLECTION CONSIDERATIONS

When it comes to galactosemia screening a simple spot of blood is the difference between life and death for an infant. How can you make sure that the blood spot you collect is adequate? Consider the following points.

Timing is everything! First specimens should be collected when an infant is between two and five days of age, or within four hours of discharge from a hospital. Collecting a blood spot specimen too early or too late can interfere with galactosemia results.

Information Please. The follow-up program uses the demographic information supplied on the screening form to locate infants with positive screens. Please make sure this information is as complete and accurate as possible!

Important dates. Forgetting to put specimen collection dates on the newborn screening form delays testing. Make sure the collection date is filled in.

It's in the mail. We can't screen the infant if we don't have the blood spot! Mail specimens as soon as they are dry.

Beat the heat! The enzyme detected in galactosemia screening is sensitive to excessive heat and/or humidity. Take care not to expose the specimen to extreme temperatures.

Whose blood are we testing? Blood transfusions alter galactosemia results because donor blood instead of infant blood is screened. Whenever possible, collect the first screening specimen before transfusing the infant.

DID YOU KNOW?

- *One in 60,000 newborns will have galactosemia. Many more will be identified as carriers of a galactosemia variant.*
- *In 2003, twenty-three infants were detected by Utah newborn screening who needed treatment for a galactosemia condition.*
- *If galactosemia is untreated severe irreversible liver and brain damage can occur. Newborn screening identifies infants before signs and symptoms are present.*
- *Galactosemia means "galactose in the blood".*

WHAT IS GALACTOSEMIA?

BY DR. NICOLA LONGO, MD, PH.D.
DIRECTOR OF METABOLIC SERVICES
UNIVERSITY OF UTAH, PEDIATRIC MEDICAL GENETICS

Galactosemia is an autosomal recessive disorder of carbohydrate metabolism caused by deficiency of enzymes in galactose utilization. The most common and clinically relevant is transferase deficiency galactosemia caused by a deficiency of galactose-1-phosphate uridyl transferase (GALT). See Figure 1. As a result of this deficiency, galactose-1-phosphate, galactose and its derivatives galactitol and galactonate accumulate in body fluids and tissues with consequent injury to the liver, kidney, and brain. In addition, galactose and other sugars are important in protein glycosylation, an essential function that allows cells to grow and communicate with the environment. Abnormal protein glycosylation has been detected in patients with galactosemia and might explain abnormal brain and ovarian development.

"Galactosemia affects all races and ethnicities, although the frequency of the disease differs among them"

Clinical presentation. Newborns on breast milk or normal infant formula receive up to 20% of their calories as lactose, a dimer of glucose and galactose. With this diet, poor feeding, vomiting, diarrhea, jaundice, lethargy progressing to coma, abdominal distention with hepatomegaly can present a few days after birth (usually at 3-14 days of age) followed by progressive liver failure. Patients with galactosemia are at increased risk for *Escherichia coli* or other gram-negative neonatal sepsis. When the diagnosis is not made at birth, liver disease and brain damage (mental retardation) may become irreversible. For this reason, galactosemia is routinely screened for in the neonatal period in most countries. Poor weight gain and cataracts can also be detected in patients surviving the neonatal period.

Frequency and genetics. Transferase deficiency galactosemia has a frequency of 1 in 60,000. It affects all races and ethnicities, although the frequency of the disease differs among them. The GALT gene localizes to 9p13 and different point mutations have been identified in patients with galactosemia. Most Caucasians have no detectable enzyme activity in either liver or red blood cells (Q188R, 70% of G alleles). Other common mutations are K285N, L195P, Y209C, IVS2-2A>G, and T138M, all of which cause a relatively severe phenotype. The Duarte (D, N314D) variant exhibits diminished red cell enzyme activity that is of unclear clinical significance. In most centers, including Utah, DG patients who have about 25% of normal activity are treated with galactose restriction in the first year of life. DD patients with 50% of normal activity are not treated and have no symptoms of galactosemia. When N314D is associated with a polymorphism (L218L), the enzyme has higher expression resulting in increased activity (LA variant). Some African-American patients lack transferase activity in erythrocytes, but retain some enzyme activity in liver (S135L) resulting in a milder phenotype. These patients have usually a better outcome than patients with classic galactosemia. There are at least 200 more rare mutations, including full gene deletions.

Diagnosis. Routine laboratory tests might indicate liver disease (increased transaminases, elevated PT/PTT, hyperbilirubinemia) and occasional mild acidosis. Plasma amino acids and urine organic acids indicate liver disease (elevated phenylalanine, tyrosine, and methionine; elevated p-OH-phenylorganic acids). Impaired renal tubular function can be detected (generalized aminoaciduria, glycosuria). There is no role for reducing substance (by Clinitest) in urine. Diagnosis requires the demonstration of deficient activity of galactose-1-phosphate uridyl transferase in erythrocytes and accumulation of metabolites (red cell galactose-1-phosphate, urine galactitol). DNA studies are useful because the enzyme assay can be hard to do accurately and for complete characterization of partial deficiencies.

Treatment and outcome. Elimination of galactose from the diet reverses growth failure, renal and hepatic dysfunction, and cataracts. Early diagnosis and treatment have improved the prognosis of galactosemia. However, patients can still have ovarian failure (primary or secondary amenorrhea), mental retardation, speech

dyspraxia, ataxia, and learning disabilities. The mechanism for these problems remains unknown, although there is some correlation with genotype, with complications being more frequent in Q188R homozygotes. The most likely mechanism for these problems is abnormal cell growth and communication during intrauterine life due to abnormal membrane glycosylation. Diet must be continued for life. Patients are periodically monitored by measuring levels of galactose-1-phosphate that remains always abnormally high in patients with classic galactosemia. This is because, even in the absence of dietary galactose, minimal amounts of the sugars are produced by the human body. Patient with DG variant galactosemia are treated with dietary restriction of galactose in the first year of life. After that, they are challenged with a diet containing known amounts of galactose. The challenge is passed if they have normal levels of galactose-1-phosphate after this challenge. These patients usually have no sequelae due to the variant form of galactosemia.

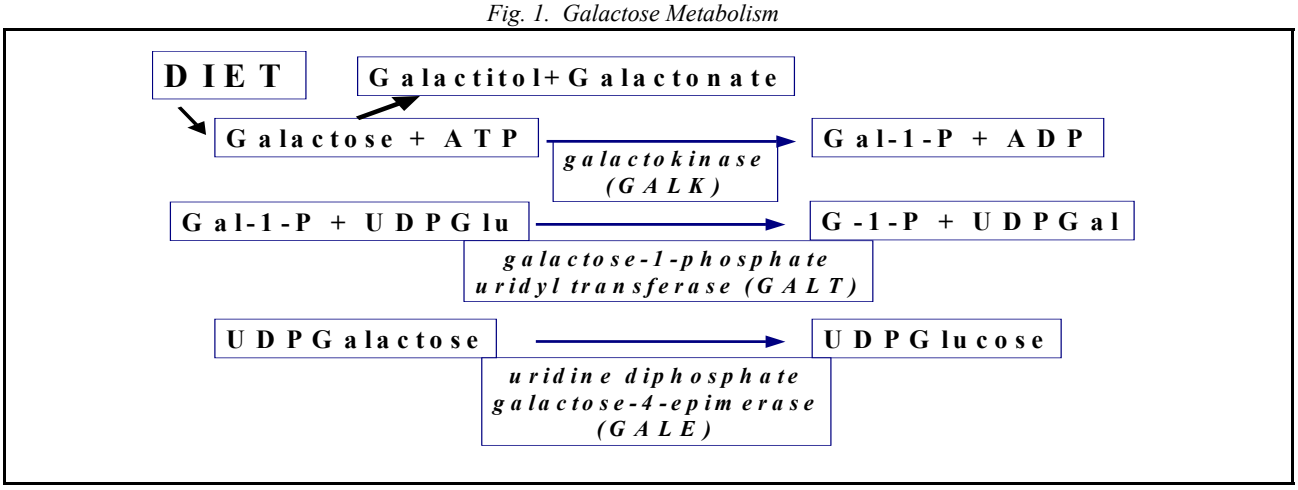
References

Elsas LJ, Lai K. 1998. The molecular biology of galactosemia. *Genet Med* 1:40-8.

Leslie ND. 2003. Insights into the pathogenesis of galactosemia. *Annu Rev Nutr*. 23:59-80.

Tyfield L, Reichardt J, Fridovich-Keil J, Croke DT, Elsas LJ 2nd, Strobl W, Kozak L, Coskun T, Novelli G, Okano Y, Zekanowski C, Shin Y, Boleda MD. 1999. Classical galactosemia and mutations at the galactose-1-phosphate uridyl transferase (GALT) gene. *Hum Mutat* 13:417-30.

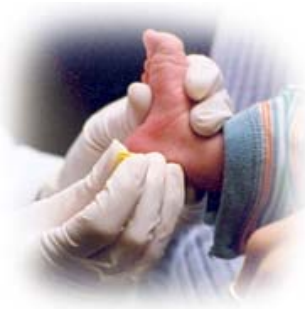
Fig. 1. Galactose Metabolism



SCREENING FOR GALACTOSEMIA

BY JANA COOMBS, RM(AAM), SV, M(ASCP)
CHIEF, METABOLIC NEWBORN SCREENING LABORATORY
UTAH DEPARTMENT OF HEALTH

Galactosemia is a rare, but potentially life-threatening, hereditary disorder of carbohydrate metabolism that affects the body's ability to convert galactose to glucose. Galactosemia means "galactose in the blood". Glucose can be used as a source of energy by the body, but galactose needs to be further broken down to glucose by a specific chemical (enzyme) before it can be utilized. The enzyme that is reduced or missing in classic galactosemia is called galactose-1-phosphate uridyl transferase (GALT).



It is generally accepted that A. Von Reuss made the first report of galactosemia in 1908. He observed an infant with an enlarged liver and spleen who was breast-fed but could not gain weight. The presence of galactose was also found in the urine (galactosuria). The infant ceased to excrete galactose in the urine when given a milk substitute; however, the child died at three weeks in the hospital. By 1917, "galactosuria" was a broadly recognized inherited disorder and was treated by removal of milk products from the diet.

Although clinicians recognized galactosemia early in the century, the defective gene that caused it wasn't found until 1956 (and wasn't fully sequenced until 1992). In 1965, E. Beutler, M.C. Baluda, P. Sturgeon, and R. Day described patients with variations in the levels of enzyme, and proposed the different levels of enzyme were due to different genotypes.

Another major break-through happened when galactosemia was first found to be detectable through a newborn screening method in 1963. Guthrie and Paigen

developed this method. Galactosemia was the second disorder found to be detectable through newborn screening methods by Robert Guthrie, with phenylketonuria (PKU) being the first.

The Utah Public Health Laboratory Newborn Screening section uses the Neonatal GALT Test Kit produced by PerkinElmer Life Sciences. This kit is intended for the semi-quantitative determination of GALT in blood specimens, dried on filter paper, as an aid in identifying newborns lacking GALT enzyme activity.

The assay is an adaptation of the Beutler and Baluda procedure. It is based on the enhancement of the fluorescence of NADPH through a series of enzymatic reactions. See figure 2. The enzymes shown above the arrows are found in the sample itself. All others are either products of the previous reaction or found in one of the reagents. The detection limit is 1.3 GALT Units/gHb.

Standards and controls, containing known concentrations of GALT, are reacted simultaneously with patient samples during each assay. The standards are used to generate a curve from which patient results are calculated. Controls assure the day-to-day validity of the results.

As with any other diagnostic test, data obtained with the screening procedure should be used as an aid to other medically established procedures, and should be interpreted in conjunction with other clinical data available to the physician. Confirmation of positive newborn screening test results is always necessary. Family studies may be necessary to determine the genotype. The potential for symptomatic diagnosis of Galactosemia is fair. Symptoms may occur before receiving the results of newborn screening, and require a high index of suspicion. In one survey, two thirds of infants were symptomatic and under care at the time of the report of the positive newborn screening result. A galactose-free diet and supportive care for *Escherichia coli* sepsis, hypoglycemia, liver failure, and coagulation problems should be instituted pending confirmation of the diagnosis.

"Confirmation of positive newborn screening test results is always necessary"

Fig. 2. Summary of reaction sequence

1. Galactose-1-Phosphate + Uridine 5'-Diphosphoglucose —Galactose-1-phosphate uridyl transferase (GALT)→ Glucose-1-Phosphate + Uridine Diphosphogalactose

2. Uridine Diphosphogalactose —Phosphoglucomutase→ Glucose-6-Phosphate

3. Glucose-6-Phosphate + Nicotinamide Adenine Dinucleotide Phosphate —G-6-PDH→ 6-Phosphogluconate + *Nicotinamide Adenine Dinucleotide Phosphate (reduced)*

4. 6-Phosphogluconate + Nicotinamide Adenine Dinucleotide Phosphate —6-PGA Dehydrogenase→ Rubulose-5-Phosphate + *Nicotinamide Adenine Dinucleotide Phosphate (reduced)*

**fluorescent compound*

FOLLOW-UP OF ABNORMAL SCREENING RESULTS

BY ANGIE LIVINGSTON, RN, BS
FOLLOW UP PROGRAM NURSE
UTAH DEPARTMENT OF HEALTH

Every year in Utah over 50,000 babies are born. Utah law requires two newborn screens be collected for each baby. Every day, in hospitals and clinics around the state, newborn screens are collected. The baby's foot is poked and the blood carefully applied to special filter paper. The specimen is air-dried and then mailed to the state lab.

Most of these specimens will test normal. Likely the results will be received, noted, filed in the chart, and then forgotten. But everyday, to medical homes across the state, "the call" is made. "This is the Utah Department of Health Newborn Screening program with an abnormal result". For these medical homes, and the families, newborn screening is not forgotten.

A positive screen for galactosemia sets into motion a chain of events that ultimately ends with diagnostic testing to confirm the abnormal screen. The newborn screening follow-up program works closely with medical homes and with metabolic consultants to make sure that confirmation testing is completed and, if needed, treatment began.

Notifying the medical home of the infant about the abnormal galactosemia screening result is the first priority for the follow up program. You can help in this process by making sure specimen cards are filled out completely and accurately. If medical home information

is missing or incorrect confirmation testing is delayed until this information can be found.

After the medical home is alerted to the positive screen confirmation testing is arranged. The medical home and the newborn screening program work together to arrange collection of blood specimens for confirmation testing. Confirmation testing is needed to make a correct diagnosis and to insure proper treatment for the infant. Whole blood specimens are collected from the infant and then sent to sent to the University Children's Genetic Laboratory in Los Angeles where galactose 1-phosphate uridyltransferase electrophoresis and galactose 1-phosphate uridyltransferase quantitative testing is completed. Blood specimens must be handled carefully after collection and during shipping to insure testability of the specimen. Specific verbal and written instructions are provided to medical homes by the newborn screening follow up program. Please follow these instructions carefully as improperly handled specimens delay results.

"A positive screen for galactosemia sets into motion a chain of events"

Having a child with an abnormal screen can be very frightening to parents. The newborn screening program can assist in educating the parents about the abnormal screen and galactosemia in general. Please inform the newborn screening follow-up nurse of any educational needs the family might have.

Confirmation testing results are available a week after the specimen is shipped. While testing is being done, it is recommended that the infant be placed on a soy-based diet to limit the amount of galactose consumed. As soon as results are available the follow-up program nurse notifies the medical home. If treatment is necessary a referral is made to the Metabolic Clinic. Successful follow-up for abnormal screens requires teamwork between hospitals, medical homes, and the newborn screening program. We thank you for the vital role you play in the follow-up of these infants.

DIETARY MANAGEMENT OF GALACTOSEMIA

BY SHARON L. ERNST, MPH, RD, CD & NANCY MCMILLAN, RD, CNSD
METABOLIC NUTRITIONISTS
UNIVERSITY OF UTAH, MEDICAL GENETICS

Classical galactosemia results from the deficiency of galactose-1-phosphate uridyl transferase. Without this transferase, galactose-1-phosphate cannot be metabolized into glucose-1-phosphate, causing a build-up of galactose-1-phosphate. The level of ensuing galactose-1-phosphate depends on several factors including galactose intake with the diet, individual internal galactose production, and the actual galactose-1-phosphate uridyl transferase activity available. Galactose-1-phosphate accumulation can be minimized by maintaining a diet that contains little galactose.

While it is impossible to eliminate all galactose from the diet, reduction in galactose intake can first be modulated by avoiding lactose. Because lactose is a disaccharide containing glucose and galactose, all milk products are unacceptable on a galactosemia-restricted diet. As soon as an infant is diagnosed or suspected to have galactosemia, he or she should be placed on a soy-based or casein hydrolysate-based infant formula. See figure 3 for the galactose content of several infant formulas.

Like regular infant formulas and breast milk, children with galactosemia should also avoid other obvious sources of lactose. Unfortunately, lactose can sometimes be hidden in food. Label reading is needed to avoid intake of casein, caseinate, dry milk solids, curds and whey in processed foods. These foods substances and others containing lactose should be eliminated in the diet and are listed in figure 4.

Foods containing lactate, lactic acid and lactylate are **not** sources of lactose and can be safely consumed. Some margarines do not contain milk products and are okay to use. Sherbet is also a safe food if it does not contain dry milk like many sorbets. To be sure if a food is acceptable on a galactose-restricted diet, careful label reading is a necessity.

Because galactose-containing components are incorporated into cell walls, foods that do not contain lactose may still be high in galactose. For this reason, garbanzo beans are eliminated in the diet and other legumes should be consumed carefully. Kidney beans, lima beans and lentils should only be used in moderation depending on individual galactose tolerance. Fermented soy products (fermented soy sauce, miso, tempe) are not recommended as galactose can be released in the fermentation process. Non-fermented soy products (tofu and soybean extract) are

"As soon as an infant is diagnosed or suspected to have galactosemia, he or she should be placed on a soy-based or casein hydrolsate-based infant formula".

acceptable. Other foods such as tomatoes, dates, papayas, bell peppers, and watermelon contain moderate amounts of galactose. These foods, however, can be included in a diet with moderation based on individual tolerance and careful monitoring by a dietitian or physician.

Many medications and vitamin and mineral supplements may also contain lactose or galactose. To determine if a drug contains lactose, you may contact the Drug Information Center at the University of Utah, 801-581-2073.

While diet cannot alleviate all the symptoms or complications of galactosemia, diet can improve or eliminate many of the symptoms and complications. Dietary philosophy used to treat galactosemia revolves around providing consistent, tolerable amounts of galactose in the diet. Patients with galactosemia keep diet records of food intake in conjunction with blood tests to determine galactose-1-phosphate levels. Diet modifications can then be made by the dietitian based on blood levels of galactose-1-phosphate. Growth and vitamin and mineral intake are also assessed to ensure diet adequacy.

As patients grow, soy-based infant formulas may continue to be the major source of calcium and vitamin D in the diet. Milk substitutes made from soy protein isolates, designed for older children and adults can also be used. Patients weaned from an infant formula and following a galactose-restricted diet often must supplement their diet with calcium and vitamin D. All calcium supplements should be checked carefully to ensure they do not contain lactose or galactose. Other vitamin and mineral supplementation may be needed if intake is not sufficient.

Careful and constant dietary management is used as the main treatment of the disease and results in improved outcomes. Diet therapy is used as a lifetime treatment. While exclusion of lactose is the first step in dietary management, often more extensive and careful dietary restrictions are needed to maintain acceptable galactose-1-phosphate levels. Normal growth and development can be obtained, but may require vitamin and mineral supplementation in addition to a healthy diet. In summary, dietary modification in conjunction with blood levels of galactose-1-phosphate can successfully treat galactosemia.

FORMULA	GALACTOSE CONTENT (mg/L)	SOURCE OF GALACTOSE
Human Milk	3,500	Lactose
Similac RTF	3,650	Lactose
Enfamil RTF	3,680	Lactose
Similac Lactose Free	< 75	Milk Protein Isolate
Enfamil LactoFree	20	Milk Protein Isolate
Isomil RTF	14	Soy Protein Isolate
ProSoBee	<4	Soy Protein Isolate
Alimentum	<37	Casein Hydrolysate
Pregestimil	<8	Casein Hydrolysate
PediaSure	355	Sodium Caseinate, Whey Protein

Fig. 3. Galactose content of selected infant formulas

Butter	Buttermilk and solids	Sherbet
Milk and milk solids	Casein or Curds	Nonfat milk
Garbanzo beans	Cheese	Lactoglobulin
Sour cream	Cream	Sodium caseinate
Lactalbumin (milk albuminate)	Dry milk	Ice cream
Organ meats (liver, heart, etc.)	Margarine	Lactose
Milk protein	Whey and whey solids	Calcium caseinate
Milk chocolate	Nonfat Milk	Yogurt
Hydrolyzed protein made from casein or whey	Nonfat dry milk and milk solids	

Fig. 4. Foods containing lactose

"While diet cannot alleviate **all** the symptoms or complications of galactosemia, diet can improve or eliminate **many** of the symptoms and complications"

GENETICS OF GALACTOSEMIA

BY BONNIE JEANNE BATY, MS, CGC
GENETIC COUNSELOR
UNIVERSITY OF UTAH, MEDICAL GENETICS

How is galactosemia caused? Galactosemia is caused by a mutation (change) in the gene that makes GALT. Genes are located on our chromosomes and contain the code for making enzymes and other products. The *gene* for the enzyme GALT is located on the short (p) arm of chromosome 9, at bands p13. *Mutations* (changes) in this gene cause the enzyme to work poorly or not at all and galactosemia results. There have been over 150 different mutations found in the *GALT* gene. Individuals with mutations in this gene can't break down galactose correctly and galactose-1-phosphate and related substances build up in the body, causing the symptoms of galactosemia. Individuals with classic galactosemia have two mutations, one from each of their parents. Individuals with Duarte galactosemia have a specific mild mutation called N314D along with another variable galactosemia mutation.

Chromosome 9

What tests are available to diagnose and treat galactosemia? Galactosemia is usually suspected with a newborn screening test, which measures the approximate amount of the enzyme GALT. It is confirmed by exact measurement of GALT and a DNA test, which identifies which *GALT* mutations are present. When the galactose level is too high, galactose-1-phosphate (Gal-1-P) accumulates. We measure Gal-1-P to make treatment decisions.

What are the chances of having another child with galactosemia? When a couple has a child with classic galactosemia, they are at 25% risk to have another child with galactosemia with each pregnancy. See Figure 5. We inherit one copy of each gene from our mother and one from our father. Children with classic galactosemia inherit two copies of a *GALT* gene mutation, one from each parent. Each parent is a carrier. Carriers of galactosemia do not have galactosemia, but have one normal GALT gene and one abnormal GALT gene. The normal gene gives them enough normal enzyme so that they don't have symptoms. Since children with galactosemia have two abnormal *GALT* genes, they have little or no normal enzyme. About 1 in 100 people are carriers of a severe *GALT* mutation. There are also some mild mutations that can cause the milder forms of galactosemia.

Is there a carrier test for galactosemia? We can do carrier testing by testing for the *GALT* mutations in the family and/or testing the GALT enzyme.

What do we recommend for other pregnancies? It is important to make sure siblings are adequately screened or receive prenatal testing for galactosemia. If prenatal diagnosis is not done, the newborn should have enzyme activity measured (in addition to the normal newborn screening) and should remain on soy formula until results are available. Please contact the Utah Department of Health newborn screening program at (801)584-8256 to arrange special testing for any future children.

What reproductive options are available? There are several reproductive alternatives available to parents at 25% risk of having a child with galactosemia. These include birth control, permanent sterilization procedures, adoption, prenatal diagnosis (amniocentesis or CVS), artificial insemination by donor, egg donation, and preimplantation diagnosis. Of course, many parents elect to have other pregnancies without any procedures.

Is there a support group? There is a support group available for galactosemia:

Parents of Galactosemic Children
20981 Solano Way
Boca Raton, FL 33433
407-852-0266
Contact: Linda Manis

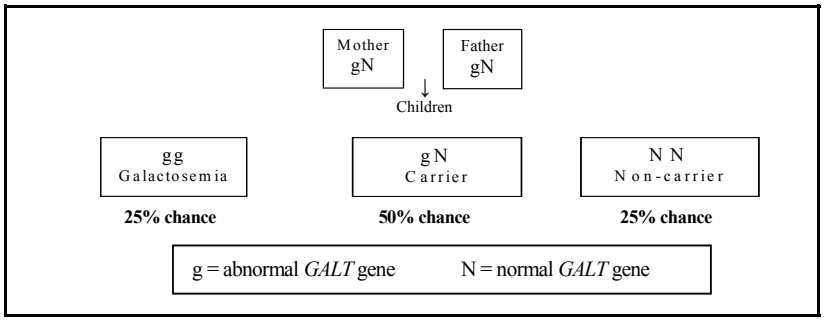


Fig. 5. Marriage of two carriers for galactosemia

A Newsletter of the Newborn Screening Program and
the Newborn Screening Laboratory
Utah Department of Health

Newborn Screening Follow Up Program
44 N Medical Drive
PO Box 144710
Salt Lake City, UT 84114-4710

Phone: 801-584-8256
Fax: 801-584-8496
Email: Alivingston@utah.gov

health.utah.gov/newbornscreening

Newborn
Screening Program

For more information on galactosemia and newborn screening check out the following websites!

- health.utah.gov/newbornscreening
- www.galactosemia.org
- http://www.tdh.state.tx.us/newborn/handbook.htm
- http://depts.washington.edu/transmet/
- http://www.savebabies.org/diseasedescriptions/galactosemia.php

Utah Department of Health
Division of Community and Family
Health Services
Newborn Screening Program
Box 144710
Salt Lake City, Utah 84114-4710